Sveriges lantbruksuniversitet
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Fodrets effekt på syrsors näringsinnehåll och anatomi

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SUMMARY

This study evaluate the chemical composition of Cambodian field crickets' (*Teleogyrus testaceus*) fed four different diets. Crickets' from an earlier study (Miech *et al*., 2016) on growth and survival was used in the study. Amino acid composition, mineral content, weight and size were analyzed. Crickets' fed Cassava plant tops were heavier and contained higher levels of several essential amino acids than the control group fed commercial chicken feed. Minerals connected to the most common mineral deficiency diseases in humans (such as iron, magnesium and zinc) did not differ between the groups.

The study also examined the possibilities to use computer tomography technique (CT-scanning) to estimate the size and volume of a crickets' gastrointestinal tract. It was possible to produce an image of part of the gastrointestinal tract using contrast but more research is needed to develop the method if it is to be used to estimate the volume of the entire gastro-intestinal tract on several crickets as it turned out to be a delicate and time-consuming task.

As a final part of the study the possibility to alter the size and volume of a crickets' gastro-intestinal tract by different diets should be investigated. This final experiment could not be completed due to high mortality among the crickets' used in the study.

SAMMANFATTNING

Denna studie utvärderar den kemiska sammansättningen hos kambodjanska syrsor (*Teleogyrus testaceus*) uppfödda på fyra olika dieter. Syrsor från en tidigare studie (Miech *et al.,* 2016) om tillväxt och överlevnad användes i studien. Aminosyrakomposition, mineralinnehåll, vikt och storlek analyserades. Syrsor uppfödda på Cassava-plantor var tyngre och innehöll högre nivåer av flera essentiella aminosyror än kontrollgruppen som fötts upp på kommersiellt kyckling-foder. Mineraler kopplade till de vanligast förekommande minerala bristsjukdomarna hos människa (såsom järn, magnesium och zink) skilde sig inte mellan grupperna.

Studien undersökte också möjligheterna att använda datortomografiteknik (CT-skanning) för att uppskatta storleken och volymen på en syrsas gastrointestinalkanal. Det var möjligt att producera en bild av en del av gastrointestinalkanalen med hjälp av kontrast, men mer forskning är nödvändig för att utveckla denna metod, om den skall användas för att beräkna volymen av hela gastrointestinalkanalen på flera olika syrsor eftersom det var en tidskrävande metod.

Som en sista del av studien skulle möjligheten att påverka storleken och volymen av en syrsas gastrointestinalkanal med hjälp av olika dieter undersökas. Detta sista experiment kunde inte fullföljas på grund av hög mortalitet bland syrorna som användes i studien.

CONTENT

INTRODUCTION

This study investigates how different feeds effect crickets' anatomy and nutritional content. It is important to gain an understanding on how the feed affect the animal to be able to use the animals in the most efficient and sustainable way.

Food production and food security is currently a much discussed subject for several reasons. Starvation and malnourishment exist in various scales on all continents. It's estimated that 795 million of the world population suffers from starvation and undernourishment (The state of Food Insecurity in the World 2015, SOFI 2015).

Meat is a food source rich in protein and including a well-balanced combination of essential amino acids needed for growth and healthy development (National food agency, Sweden. 2016). This is especially important for growing children. However todays methods of producing meat have a large impact on the environment as it is one of the most recourse demanding industries in the world (Gerber *et al*., 2013; Godfray *et al*., 2010). Large amounts of water, land, energy, antibiotics, and pesticides are used by the livestock industry chain (Gerber *et al*., 2013; Godfray *et al*., 2010). In addition the meat produced does not reach those in greatest need of nutritious food. Many people in need of nutritious food to gain health and strength to rise themselves and their society from poverty cannot afford meat.

Therefore, there is a need for an alternative to conventional meat production such as beef, pork and poultry.

Farming insects both for human consumption and animal feed is considered to be a possible solution of or at least part of a solution to this humane and environmental crisis (van Huis *et al.,* 2013). Ideally it would be possible to use discarded materials and bio-waste from other industries and use them as feed in insect farming thereby transforming waste into high value food and feed recourses.

The chances of creating an environmentally friendly, affordable and effective model for insect farming increases naturally by increased knowledge of the insects. There are already several studies on the subject of practical cage design, feed, temperature, hatching and raising (Nakagaki *et al*., 1991; Clifford *et al*., 1990; Roe *et al*., 1980; Ghouri and McFarlane., 1958.). This study focus on feed and the effect it has on crickets raised for food production.

It has recently been shown that Cambodian field crickets (*Teleogryllus testaceus*) can grow equally fast on some common weeds and agricultural by-products as on commercial chickenfeed (Miech *et al*., 2016).

To make sure the crickets fed weeds and by-products are equal as a food product to the ones fed chicken feed we are in this study analysing some of the main nutritional markers (amino acids, minerals, ash) and comparing groups fed different weeds and agricultural by-products to one control group fed chicken feed.

In some European countries it is recommended to remove the legs and wings before consumption to reduce the possible risk of chocking (van Huis *et al.,* 2013). The wings and legs was therefore analysed separately from the body in the present study in order to investigate if there is a difference in the protein and mineral content. If these removed body parts are highly nutritious these recommendations might prove a terrible waste.

In this study we also wanted to investigate if the size and shape of a crickets' gastrointestinal tract can be altered by using different feed.

Studies of birds have shown that the anatomical structure, size and weight of the birds gastrointestinal tract changed depending on their diet (Lewilv, 1963). A diet with high fibre content resulted in a longer and heavier food track. The authors of the article describes the changes to be a result of a thickening of the lining of the walls and increased muscle mass around the intestines due to the harshness of the feed compared to a diet with less fibre (Pendergast *et al.,* 1972). If there exists a similar relationship between the size of the gastrointestinal track and the feed in crickets as well as in birds it might prove useful knowledge in the search for the most appropriate feed to grow large, nutritious crickets sustainably.

The anatomy of various species of crickets have been examined trough dissection (Biagio *et al*., 2009; Woodring *et al*., 2007). As an additional part to this study we also examined the possibility to use Computer Tomography to create a three dimensional image of a crickets gastrointestinal track in order do estimate its size and volume.

LITERATURE REVIEW

Entomophagy: Insects as human food

Insects has for a very long time been used as food in many cultures across the world especially in Africa, Asia, Australia and Latin America (FAO, 2013). They are thought to supplement the diet of about 2 billion people (Bukkens 1997). Termites, crickets, grasshoppers, locusts, beetles, ants, bees and moth larvae have been among the most frequently consumed and some species are even considered a delicacy (Godfray *et al.,* 1994).

Insects as animal feed

Large amounts of land and water is used for growing traditional livestock feed and it's well- known that the livestock supply chain is a major contributor to climate change (FAO 2006; Gerber *et al.,* 2013). Large amounts of protein are necessary to raise animals as quickly as is done in modern commercial systems. Using insects as protein source in animal feed may be a cost-effective and environmentally friendly alternative to products like soybeans and fishmeal witch is often used today. Studies show that palatability is high and depending on the insect species in the feed and animal species consuming the feed, 10-100% of fishmeal or soymeal can be replaced (Wang *et al.,* 2005; Liu and Lian, 2003; Hale, 1973 cited by Newton *et al.,* 2005). Using insects to convert organic waste such as manure, soiled fruit and vegetables to high value materials like food and biodiesel is also clearly a matter worth investigating (Makkar *et al,*. 2014)

The following text focus on crickets and the potential to use this insect in insect farming.

Nutritional values

According to earlier studies crickets contain protein and amino acids as well as vitamins and also several minerals important for human development and health (Table 1, 2 and 3).

Table 1. *Chemical composition of house cricket*

Crude protein (% in DM)	Neutral detergent fibre	Acid detergent fibre (% in DM)	Ether extract (% in DM)	Ash (% in DM),
63.6±5.7	18.3±2.9	10.0	17.3±6.3	5.6±2.4

Sources: Barker *et al.* (1998) and Finke 2002 cited by Makkar *et al*. (2014).

Table 2**.** *Mineral content of house cricket (all values in g/kg DM except Cu, Mn, Fe and Zn which are in mg/kg DM)*

Ca,	P	Mg	Cu	Mn	Fe	Zn
10.1±5.3	7.9	2.1	15.0±7.0	40.0±10.0	116.0±58.0	215.0±60.0

Source: Finke 2002 cited by Makkar *et al*. (2014).

Table 3. *Amino acid composition of field cricket*

Amino acid	g/16 g nitrogen
Alanine	15.6
Arginine	3.7
Aspartic acid	6.3
Cystine	1.0
Methionine	1.9
Lysine	4.8
Isoleucine	3.1
Leucine	5.5
Phenylalanine	2.9
Threonine	2.8
Glutamic acid	9.1
Histidine	1.9
Proline	4.5
Serine	3.7
Glycine	3.6
Tyrosine	3.9
Valine	4.4

Source: Wang *et al.* (2005).

Farming crickets

Catching wild crickets posts a potential threat to the wild populations and according to FAO (FAO, IFAD and WFP, 2015. SOFI 2015) a key factor in reducing under-nourishment and hunger in the world is economic growth but only when it is inclusive and by other words is providing opportunities for the poor to improve their livelihoods. Therefore ways to farm crickets has recently been investigated and in particular sustainably, low cost methods that include both feed and choice of species used in the production (Collavo *et al*., 2005).

Cages and rearing facilities

Water consumption in crickets varies between 28-100 mg water/cricket/day depending on age and temperature during their last larval stage (Roe *et al*., 1980). Studies in the common house cricket *Acheta domestiucus* shows that they can be bred in small, easy to build containers as long as the humidity can be regulated to 100% for eggs and about 50% for older crickets. Cardboard cartons fitted with a screen lid, aquaria, vented plastic cages, candy jars and wooden boxes with strips of aluminium foil glued to the upper surface have all been used. Crickets cannot climb smooth surfaces and can thus fairly simple be kept without escaping even without a tight fitting lid (Nakagaki *et al*., 1991; Clifford *et al*,. 1990; Roe *et al*., 1980). The conditions for reproduction are not considered difficult. Jars containing soft damp material such as sand, peat moss, have been used successfully in several studies to encourage females to oviposit. (Clifford *et al*., 1990; Ghouri and McFarlane., 1958) The insects reproduce quickly. One female can produce on average 2994 eggs during a lifetime and the offspring grows quickly. In ultimate rearing conditions crickets mature and are ready to harvest for consumption in about 50 days from egg to adulthood (Clifford *et al*,. 1990). By using species innate to the area the animals are farmed in the risk of introducing foreign pests that is a potential threat to the local ecosystem is eliminated.

Feed

Crickets bred as research animals, fish bait or food for exotic pets and zoo animals are often raised on chicken feed or in some cases specialised diets (Hanboonsong *et al*., 2013; Lundy *et al*., 2014; Nakagaki *et al*., 1991). It would make the production more cost-effective and environmentally friendly if the animals were raised on discarded materials such as different kinds of bio waste. One example suggesting this is possible a recent study were it is shown that Cambodian field crickets (*Teleogryllus testaceus*) can grow as quickly and as much on some common local weeds as on commercial chickenfeed (Miech *et al*., 2016).

Diseases

There's a great deal of optimism regarding the future of cricket farming however there have been outbreaks of virus infections in cricket farms causing high mortality rates. In 1980's there was an outbreak of a rhabdovirus-like infection in USA with more than 80% mortality. The infection seemed to spread via eggs and cannibalism. The crickets showed signs of lowered activity, twitching and shaking (Adams *et al*., 1980).

In 2009 house cricket breeding in North America was decimated because of outbreaks of densovirus (AdDNV). It later spread outside the continent and today breeders in other parts of the world are affected as well. The different size and genome of the virus indicate that it might be a new virus family or genus of which much is unknown (Pham *et al*., 2013).

The European house cricket, *Acheta domesticus*, has proven to be very susceptible to *A. domesticus densovirus* (AdDNV). The mortality is predominant in the last larval stage and among young adults. Symptoms include dwarfism, reduced activity, and a lifespan shorter than 15 days in adult females (Szelei *et al*., 2011). A completely empty digestive caecae is the most obvious pathological feature and infected tissues included adipose tissue, midgut, epidermis, and Malpighian tubules (Szelei *et al*., 2011). The North American sequence differs slightly from the European (Szelei *et al*., 2011). Other cricket species have been experimentally introduced to the virus and do not seem to be susceptible (Szelei *et al*., 2011).

MATERIAL AND METHODS

The study was divided into three major parts: Estimation of the nutritional content in crickets fed four different diets; Using CT-scanning to create a 3-dimensional image of a cricket's gastrointestinal tract; Investigate if it is possible to alter the size and shape of a cricket's gastrointestinal track by different diets.

Estimation of the nutritional content in crickets fed four different diets

Insects and feed

The insects used in part one were Cambodian field crickets *(Teleogyrus testaceus)* acquired form an earlier study on survival and growth on 13 diets by Miech *et al*., (2016). The crickets had been captured in the wild and placed in breading colonies to produce animals of desired amount and age for the experiment. Individuals from the third generation were used in the experiment on growth and survival. Surviving insects from the study were dried at 60 ^{o} C for 36 h and frozen for preservation.

From these crickets all individuals from 4 diets were chosen for this study. The control group (chicken feed) and crickets from three groups which had gained most weight and showed good survival in comparison to the control group (the agricultural by product cassava tops and the weeds *Cleome rutidosperma* and *Synedrella nodiflora*). The chemical composition of the feeds used by Miech *et al.*, 2016 are shown in Table 4.

Table 4. *Dry matter content, chemical composition and estimated gross energy content of chicken feed, weeds and agricultural by products used in Miech et al., 2016 study as well as this study*

	Dry matter	**Crude protein**	**Ash**	**Crude fibre**	**Crude fat**	**Gross energy**
Feeds	%		- % of dry matter -			MJ/kg DM
Chicken feed *	91.1	23.4	6.5	5.7	9.0	20.0
Cleomone rutidosperma	14.3	22.2	17.3	28.3	3.2	17.5
Cassava tops	19.7	28.6	5.2	14.2	4.6	20.0
Synedrella nodiflora	11.3	19.3	16.5	21.0	4.8	17.6

*Top Feed, Pathum Thani, Thailand

Chemical analysis

The material was prepared for chemical analysis by removing wings and the large pair of hind legs (femur, tibia and tarsus) from the body by hand (Figure 1). These were analysed separately from the bodies. The entire length of the insect (including antennas and egg duct if present), the length of body and the length of the femur was recorded for each individual. The weight of the entire insect, the body, the wings and the hind legs was recorded with an electronic scale (model Sartorius 1702 MP8; capacity: 202 g, readability: 0.1mg; Uppsala, Sweden). The material was pounded into a fine powder before sent off for chemical analysis.

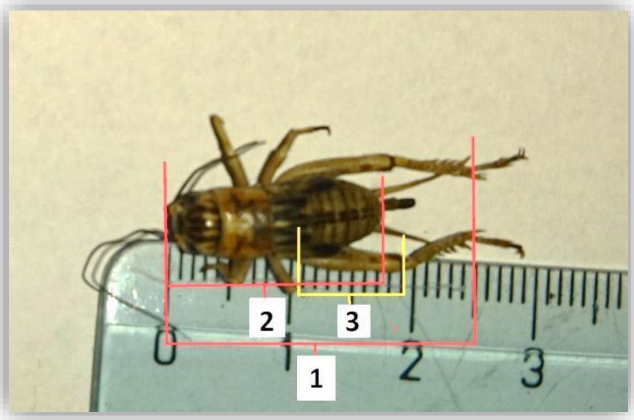

Figure 1. *The photography illustrates how the body parts were divided during measuring. 1: Total body length, 2: Body length, 3: Femur length.*

Dry matter was determined by drying the samples at 103^{o}C for 16 h and for ash after ignition at 600^{o}C for 3 h. The analysis was performed by the department of Animal nutrition and Management, Swedish University of Agricultural Science (SLU).

The amino acid analysis was performed by Eurofins Food and Feed AB using Method/ref SS- EN ISO 13903:2005. The method describes the determination of free (synthetic and natural) and total (peptide-bound and free) amino acids in materials, using an amino acid analyzer or HPLC equipment.

The mineral analysis was performed by ALS Scandinavia AB. A semi-quantitative screening analysis by ICP-SMS (HR-ICP-MS) after MW-assisted acid digestion. The method analyses the mineral content using inductively coupled plasma as an ionized sourse and high resolution mass spectrometry.

Statistical analysis

Statistical analysis was performed in SAS (version 9.4, SAS, Inst. Inc, Cary, NC) using analysis of variance and the GLM procedure. The model included: diet (4 levels) and sample (2 levels) or cricket individual number (range X-Y crickets per diet). The level of statistical significance was set to $P < 0.05$. Values are presented as LSmeans ± SEM.

Using CT-scanning to create a 3-dimensional image of a crickets' gastrointestinal tract

Insects

Crickets of unknown species were bought from a pet shop. The animals had an on average body length of 1.7 cm and an average weight of 199mg (model Sartorius 1702 MP8; capacity: 202 g, readability: 0.1mg; Uppsala, Sweden).

Preparations and contrast

The crickets were randomly divided into four groups with four individuals in each and placed in small plastic containers 10x10x5 cm with multiple air holes the size of a small pinhead on the side. Over the night they had free access to food and water (some regular pieces of fruit and a wet tissue in a small cup). Three different dilutions, 100%, 50% and 10% of barium sulfate (Mixobar®Colon, Bracco, Sweden) was prepared and mixed with a standardised amount of banana (Table 5). The food was removed 8 h before the crickets were given contrast to increase their appetite. Each mixture was placed in a cup and placed in the different cages for 2 h. A cup with only banana and water was placed in the fourth cage.

Table 5. *Proportions of barium sulfate and banana in the four treatments*

Group	Banana	Diluted Barium sulfate	Proportion contrast and water
100%	1.2765g	900µl	1;0
50%	1.2806g	900µl	1;1
10%	1.2725g	900µl	1;9
0%	1.2776g	-	0;1

After 2 h the mixtures was removed and the cages containing the crickets were placed in a refrigerator 4-6 ^{o}C to force them into hibernation and stop the passage of the contrast through the gastrointestinal tract. A pilot study preformed prior to this study, indicated that this procedure could be relevant for the detection of barium sulfate in the gastrointestinal tract.

Scanning and image processing

Five crickets, one at each time, were run through a Siemens Sematom Definition As CT- scanner. One from each group and one extra from the 10%-group as it was not enough contrast in the first 10%-cricket to create a reliable image. The insects were positioned belly down in a 90 degree angle to the x-ray tube head facing the gantry on a hard foam witch does not create artefacts and covered with thin plastic. Computer settings for the scanning is presented in Table 6.

Table 6. *Settings Siemens Sematom Definition As CT-scanner*

Program: **Can Spine Hel**	
Spineprotocoll 120 kV 400 mAs Pitch 0.8	
Construction	**Construction**
Soft tissue reconstruction	Skeletal reconstruction
Slice: 0.6	Slice: 0.6
Kernel: B 30s medium smooth	Kernel: B 70s very sharp
Window: Spine	Window: inner ear
Increment: 0.3 and 0.6	Increment: 0.3 and 0.6

The images were processed in the Osirix software (Uppsala, Sweden). Settings WL:500 and WW:1 in Osirix were used when calculating the volume of barium sulfate inside the cricket. These settings create a smooth and clear picture of the barium without the rest of the cricket. Using the ROI tool the outer region of the barium was marked in each slide of the series. Using the Calculate ROI:s volume tool for the entire series a 3D-image of the marked area was created as well as the estimated volume was calculated.

To investigate the methods reproducibility one cricket from the 10%-group was scanned three times picking up the cricket between the scans to mimic a new examination taking place. The result on the three different volume estimations on the same cricket was compared.

Effects of diet on size and shape of a cricket's gastrointestinal tract

Insects and cages

House cricket nymphs *(Acheta domesticus)*, stage 1-4 and average weight 4mg (model Sartorius 1702 MP8; capacity: 202 g, readability: 0.1mg; Uppsala, Sweden) was acquired from a specialised pet store. The crickets' were for one week kept in the same cages and room where they would be kept for the entire experiment to acclimatise. Out of these crickets 160 were randomly chosen for the experiment.

Average weight of the crickets in the beginning of the experiment was calculated as the remaining crickets were euthanized by placing them in a freezer and counted and weighed (model Sartorius 1702 MP8; capacity: 202 g, readability: 0.1mg; Uppsala, Sweden). At the end of the experiment the surviving crickets should be counted and weighed.

Eight plastic cages, 36x27x23 cm, were prepared for the experiment. The cages had holes cut on two sides (7 cm in diameter) covered with a tight mesh to provide air circulation. Each cage contained one Petri dish filled with gravel and water, a plastic flower pot cut in half for shelter and one square 8x8 cm of hard plastic foam to enable easy access to the water. The Petri dish was filled with gravel to decrease the risk of drowning. Plastic flowerpots were used as shelter instead of the usual paper cardboard egg holder to make sure the crickets were only eating the provided diet.

Room temperature was kept at 20±2^{o}C and humidity 52%. Four cages were adapted with warming mats (10x12.5 cm, Exoterra, Heat Wave, Desert, Extra small) mounted underneath to provide extra heat. Water and feed were changed every third day and excrement was removed. Temperature and humidity was observed daily using an analogue thermo- and hygrometer (Analogue thermo- and hygrometer, Dragon Terraristik Bedarf, Linköping, Sweden).

Feed and estimation of gastrointestinal size and shape

Half of the crickets were fed silage and half were fed a commercial chicken feed. Nutritional value of the two feed is presents in Table 7.

Table 7. *Nutritional value of silage and chicken feed used as cricket feed*

Feed	Dry matter % /kg feed	Crude protein g/kg feed	Gross energy MJ
Chicken feed *	93	190	11 (WPSA)
Silage	53	94	6.1 (OE-Häst)

* Pullfor Penna, Lantmännen Lantbruk, Malmö, Sweden

When the crickets had reached a larva stage 6-9 or wing pads first appear among the majority crickets from each group would be randomly selected and the size and shape of their gastrointestinal track would be investigated using either micro dissection or the CT-scan technique described in Part 2.

However, six weeks into the study the experiment was cancelled due to high mortality among the animals in the experiment.

RESULTS

Chemical composition

There was a significant difference in the composition of essential amino acids between the control group (chicken feed) and two of the experimental feeding groups, Cassava tops and *Clemone rutidosperma* (Table 8). In general there was a higher content of several diffrent amino acids in the Cassava tops group than in the other groups. There were no significant difference in amino acid composition nor in organic matter, dry matter or ash between chicken feed and the weed *Synedrella nodiflora* (Table 8).

The group fed Cassava tops contained less organic matter and ash and had a lower percentage dry matter than the chicken feed group. It also contained less of the amino acid Tyrosine. The Cassava tops group did contain significantly more of the amino acids Arginine, Cysteine and Cystine, Glutamic acid, Lysine, Methioine and Phenylalanine than the chicken feed group.

The group fed the weed *Clemone rutidosperma* contained less organic matter and less of the amino acids Alanine, Proline and Tyrosine than the chicken feed group. The *Clemone rutidosperma* group did contain more ash and more of the amino acid Phenylalanine than the chicken feed group.

There was a difference in the amount of organic matter, ash and the amino acid serine between the samples containing the insects' wings and legs and the samples containing the bodies (Table 10). The samples containing the bodies contained more ash, serine and less organic matter than the sample containing the legs and wings.

Table 8. *Dry matter (DM %), organic matter (%) ash (%) and amino acid content (g/100g DM) of Cambodian field crickets' fed chicken feed, cassava tops, and two different weeds*

	Chicken feed	**Cassava Top**	***Cleome rutidosperma***	***Synedrella nodiflora***	**P-value Feed**	**p-value Body**
DM	94.7±0.27^{a}	92.4±0.27^{b}	93.9±0.27^{a}	94.4±0.27^{a}	0.03	0.60
Organic matter	90.5 ± 056^{a}	87.5 ± 0.56^{b}	87.7 ± 0.56^{b}	89.3 ± 0.56^{a}	0.08	0.03
Ash	4.17±0.35 ^{a}	5.00±0.35 ^{a}	6.22±0.35 ^{b}	5.13±0.35 ^{a}	0.09	0.01
Alanine	8.49 ± 0.57^{a}	6.76 ± 0.57^{a}	5.47 ± 0.57^{b}	8.01 ± 0.57^{a}	0.09	0.18
Arginine	2.21 ± 0.26^{a}	3.41 ± 0.26^{b}	2.75 ± 0.26^{a}	2.23 ± 0.26^{a}	0.16	0.60
Aspartic acid	3.56 ± 0.34	4.83 ± 0.34	3.68 ± 0.34	3.22 ± 0.34	0.14	0.08
Cysteine + Cystine	0.28 ± 0.03^{a}	0.43 ± 0.03^{b}	0.39 ± 0.03^{a}	0.30 ± 0.03^{a}	0.09	0.16
Glutamic acid	3.75 ± 0.44^{a}	5.75± 0.44^{b}	4.70 ± 0.44^{a}	3.90 ±0.44^{a}	0.13	0.66
Glycine	3.88 ± 0.30	3.38 ± 0.30	2.97 ± 0.30	3.85 ± 0.30	0.28	0.48
Histidine	1.32 ± 0.11	1.36 ± 0.11	1.12 ± 0.11	1.31 ± 0.11	0.51	0.27
Hydroxyproline	X ± Y^{a}	X ± Y^{a}	X ± Y^{a}	X ± Y^{a}	-	-
Isoleucine	1.91 ± 0.19	2.22 ± 0.19	1.77 ± 0.19	1.89 ± 0.19	0.47	0.91

Leucine	3.55 ± 0.30	4.24 ± 0.30	3.37 ± 0.30	3.54 ± 0.30	0.36	0.34
Lysine	1.49 ± 0.19^{a}	2.57 ± 0.19^{b}	2.21 ± 0.19^{a}	1.75 ±0.19^{a}	0.08	0.20
Methionine	0.55 ± 0.06^{a}	0.90 ± 0.06^{b}	0.80 ± 0.06^{a}	0.54 ± 0.06^{a}	0.04	0.55
Phenylalanine	0.03 ± 0.06^{a}	0.62 ±0.06^{b}	0.57 ± 0.06^{b}	0.03 ± 0.06^{a}	0.01	0.21
Proline	4.88 ± 0.32^{a}	4.14 ± 0.32^{a}	3.36 ± 0.32^{b}	4.62 ± 0.32^{a}	0.13	0.14
Serine	2.39 ± 0.20	2.80 ± 0.20	2.18 ± 0.20	2.04 ± 0.20	0.21	0.04
Threonine	1.67 ± 0.16	1.99 ± 0.16	1.67 ± 0.16	1.66 ± 0.16	0.51	0.46
Tyrosine	4.86 ± 0.11^{a}	3.90 ± 0.11^{b}	3.43 ± 0.11^{b}	4.80 ± 0.11^{a}	0.01	0.89
Valine	3.97 ± 0.28	3.65 ± 0.28	2.95 ± 0.28	3.84 ± 0.28	0.23	0.26

Mineral composition

There was a significant difference in the mineral content between the control group (chicken feed) and all of the experimental feeding groups, Cassava tops, *Clemone rutidosperma* and *Synedrella nodiflora* regarding cobalt, chromium, copper, molybdenum and nickel (Table 9). There were no other significant differences in mineral content between the different feeding groups.

The amount of cobalt was significantly lower in the Cassava tops group than in the other feeding groups.

The amount of chromium was significantly lower in all the feeding groups than in the control group (chicken feed).

The amount of molybdenum in the Cassava tops group and the *Synedrella nodiflora* group was significantly lower than in the control group (chicken feed) and the amount of nickel was significantly lower in the Cassava top group and the *Cleomone rutidosperma* group than in the control group (chicken feed).

There was no significant difference in mineral composition between the samples containing only the bodies and the samples containing the wings and legs except for the amount of calcium. The body samples contained 2.91 times more calcium than the samples containing the wings and legs (table 10).

Table 9. *Mineral composition (all values in mg/g DM except cobalt, chromium, mercury, nickel, lead and selenium which are expressed in mg/kg DM) of Cambodian field crickets' fed chicken feed, cassava tops, and two different weeds*

	Chicken Feed	**Cassava Top**	***Clemone rutidosperma***	***Synedrella nodiflora***	**P-value Feed**	**P-value Sample**
Aluminium	0.21 ± 0.12	0.12 ± 0.12	0.43 ± 0.12	0.12 ± 0.12	0.37	0.17
Calcium	3.20 ± 0.62	1.62 ± 0.62	3.21 ± 0.62	2.44 ± 0.62	0.38	0.03
Cobalt	0.29 ± 0.04[a]	0.12 ± 0.04[b]	0.40 ± 0.04[a]	0.28 ± 0.04[a]	0.05	0.78
Chromium	2.67 ± 0.16[a]	0.73 ± 0.16[b]	1.20 ± 0.16[b]	0.99 ± 0.16[b]	0.01	0.25
Copper	0.023 ± 0.002	0.02 ± 0.002	0.02 ± 0.002	0.03 ± 0.002	0.05	0.95
Iron	0.23 ± 0.072	0.15 ± 0.072	0.39 ± 0.072	0.19 ± 0.072	0.28	0.26
Mercury	0.008 ± 0.002	0.004 ± 0.002	0.014 ± 0.002	0.006 ± 0.002	0.09	0.24
Iodine	0.03 ± 0.004	0.01 ± 0.004	0.01 ± 0.004	0.01 ± 0.004	0.08	0.91
Potassium	16.49 ± 4.64	9.04 ± 4.64	21.96 ± 4.64	24.28 ± 4.64	0.20	0.27
Lithium	0.0020 ± 0.001	0.0001 ± 0.001	0.0002 ± 0.001	0.0004 ± 0.001	0.18	0.44
Magnesium	1.81 ± 0.44	1.28 ± 0.44	1.77 ± 0.44	1.45 ± 0.44	0.81	0.52
Manganese	0.18 ± 0.03	0.16 ± 0.03	0.15 ± 0.03	0.14 ± 0.03	0.74	0.06
Molybdenum	0.003 ± 0.0002[a]	0.001 ± 0.0002[b]	0.002 ± 0.0002[a]	0.001 ± 0.0002[b]	0.02	0.58
Sodium	5.38 ± 0.98	3.03 ± 0.98	6.21 ± 0.98	5.44 ± 0.98	0.30	0.15
Nickel	1.63 ± 0.20[a]	0.55 ± 0.20[b]	0.75 ± 0.20[b]	1.93 ± 0.20[a]	0.04	0.15
Phosphorus	14.82 ± 3.33	8.58 ± 3.33	15.00 ± 3.33	15.00 ± 3.33	0.53	0.53
Lead	0.44 ± 0.14[a]	0.20 ± 0.14[a]	0.82 ± 0.14[a]	0.23 ± 0.14[a]	0.14	0.09
Sulfur	11.84 ± 2.07	5.83 ± 2.07	12.04 ± 2.07	9.51 ± 2.07	0.30	0.15
Selenium	0.72 ± 0.06	0.36 ± 0.06	0.29 ± 0.06	0.37 ± 0.06	0.08	0.38
Zinc	0.46 ± 0.12	0.17 ± 0.12	0.24 ± 0.12	0.30 ± 0.12	0.51	0.13

Table 10. *Differences in the mineral composition (mg/g DM) organic matter (%), ash (%) and amino acid composition (g/100g DM) between the samples containing the bodies and the samples containing wings and legs*

	Body	**Wings and legs**	**P-value**
Organic matter	87.64 ± 0.40	89.86 ± 0.40	0.02
Ash	6.16 ± 0.25	4.09 ± 0.25	0.01
Serine	2.70 ± 0.14	2.00 ± 0.14	0.04
Calcium	3.90 ± 0.44	1.34 ± 0.44	0.03

Anatomical data

Crickets' fed the weeds *Cleome rutidosperma* and *Synedrella nodiflora* were significantly smaller than the control group and the group fed Cassava tops (Table 11). The total length and the body length were shorter in the groups fed weeds. There was however no significant difference in the total weight between the *Clemone rutidosperma* group, the *Synedrella nodiflora* group and the chicken feed group. The crickets' from the Cassava top group were significantly heavier than the chicken feed group even though there were no significant differences in size between the groups. The wings from the insects in the chicken feed group weighed more than the other groups. There was no significant difference in the length of the femur or the aggregate weight of wings and legs between the groups.

Table 11. *Values length (mm) and weight (g) body parts Cambodian field crickets'*

	Chicken Feed	**Cassava top**	***Clemone rutidosperma***	***Synedrella nodiflora***	**P-value**
Total length	37.06 ± 1.60^{a}	36.29 ± 1.21^{a}	32.51 ± 1.05^{b}	32.61 ± 1.12^{b}	0.02
Body length	25.75 ± 0.59^{a}	24.75 ± 0.45^{a}	22.97 ± 0.39^{b}	22.79 ± 0.41^{b}	<0.0001
Femur length	14.08 ± 0.38	13.79 ± 0.30	13.50 ± 0.23	13.70 ± 0.24	0.60
Total weight	0.12 ± 0.02^{a}	0.16 ± 0.01^{b}	0.14 ± 0.01^{a}	0.11 ± 0.01^{a}	0.009
Wings and legs weight	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.93
Wings weight	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.01 ± 0.01^{b}	0.02 ± 0.01^{b}	0.03

Using CT-scanning to create a 3-dimensional image of a crickets' gastrointestinal tract

The crickets' showed mild interest in the mixture of banana and contrast and only three crickets' was with certainty observed eating the mixture. Contrast was however observed inside all crickets' run through the CT-scanner except the one from the 0% group. The second cricket in the 10% group contained noticeably less contrast than the first from the same group.

Handling the crickets' sedated by lowering their body temperature was simple. The only movement occurring during the scanning process was occasional twitching of the antennas.

Images processed in Osirix with a high resolution and low contrast created images in which the outline of a tubular, partly gas filled structure, stretching in a cranio/caudal direction was visible.

Pictures processed with high resolution and low contrast resulted in images were the outline of the gastrointestinal track was most clearly distinguishable from other structures. The subjective view was that the 10% barium sulphate-dilution created images of better quality than the other two dilutions. The high concentrations of barium sulphate in the 100%- and 50%-dilution resulted in images were the outlining of the intestine were difficult to distinguish due to the radiance of the barium sulphate.

As for calculation the volume of the barium; the result of several scans of the same cricket are shown in Table 12 and resulted in a CV of 5.8 % (mean 5.9920, SD 0.3521).

Table 12. *Calculated volume of contrast in cricket*

Sample	Calculated volume
B1 Cricket 3 10% repl. 1	Volume: 5,5996 mm^3
B1 Cricket 3 10% repl. 2	Volume: 6,2805 mm^3
B1 Cricket 3 10% repl. 3	Volume: 6,0958 mm^3

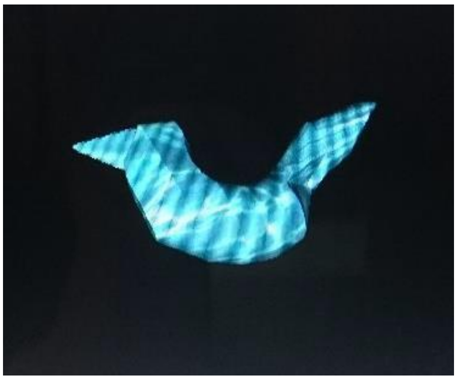

Figure 2. *3D-image of a small part of a crickets' gastrointestinal track created using*

Figure 3. *Transverse image of a cricket containing contrast 10%-solution.*

Investigate if it is possible to alter the size and shape of a cricket's gastrointestinal tract by different diets

The experiment was cancelled before any results were produced due to high mortality among the crickets' used in the study.

DISCUSSION

It has already been concluded that Cambodian field crickets' (*Teleogyrus testaceus)* fed cassava plant tops or *Cleomone Rutidosperma* had the same growth performance as crickets' fed chicken feed (Miech *et al.,* 2017). After analysing the amino acid content we can also see that the amino acid profile is equal between the groups. In the case of crickets' fed cassava tops the protein quality was even better. The one exception was the amino acid Tyrosine of which the crickets' fed cassava tops contained less of than the group fed chicken feed. This should however not cause a significant problem when used as food or feed considering that the crickets' still contain high levels of Tyrosine.

This study does, as some previous ones (Wang *et al.*,2005; Makkar *et al*,.2014) show that crickets' contain high levels of essential amino acids (Table 13 and 14). How much of these amino acids humans and other species actually can obtain from crickets' should be investigated to finally conclude crickets' value as human food and animal feed.

Table 13. *Amino acid content (g/ 16g N) in crickets (Source: Makkar et al., 2014)*

	House cricket	FAO reference protein for 2-5 year old child
Methionine	1.4	2.5 (meth. + cyst)
Cystine	0.8	
Lysine	5.4	5.8

Table 14. *Amino acid content of foods (mg/100g food) and amino acid requirements in adults (mg/kg/day)*

	Histidine	Iso-leucine	Leucine	Lysine	Methionine + Cysteine	Phenyl-alanine + Tyrosine	Threo-nine	Trypto-phane	Valine
Beef and veal[b]	603	852	1435	1573	478 226	778 637	812	-	886
Chicken[b]	525	1069	1472	1590	502 562	800 669	794	205	1018
Fish fresh all types[b]	665	900	1445	1713	539 220	737 689	861	-	1150
Crickets' feed cassava[c]	1260	2050	3920	2370	830 400	570 3600	1840	-	3370
Amino acid requirements in adults mg/kg/day[a]	10	20	39	30	10 4	25	15	4	26

a Source FAO/WHO/UNU, 2002.
b Source FAO, 1970.
c Value calculated from data acquired in this study.

The mineral analysis did show some differences between the different feeding groups. Cobalt, chromium, molybdenum and nickel are not among the most common mineral deficiencies around the world (FAO, 2001) therefore, even though the levels of these minerals were lower in some of the test groups than in the control group, this is not considered an issue. Copper was the only mineral of significance of which there was less in the test groups than in the control group. If the difference is small enough to overlook can be debated.

The results in the study indicate that the amino acid profile and mineral content of crickets' is affected by the feed and this should be taken into consideration when evaluating different feed and diets for cricket farming. This study also supports the view that it is possible to farm large, fast growing crickets' of high nutritional quality using common weeds and by-products from other industries. This can make it possible to farm crickets' in a low cost and still sustainable way.

All laboratory tests and chemical analysis was performed with very small amounts of samples due to a limited amount of research material. This raises the question of how reliable the results are. To confirm the validity of the results a similar study should preferably be done.

Since there were only a few significant differences in the samples containing the bodies and wings and legs it can be said that no valuable materials are removed when peeling (removing wings and legs) is performed for human consumption. However since the wings and legs still contained the same amino acids and minerals as the bodies they could possibly be used instead of wastefully thrown away. As an example they could be turned into flour and used as supplement or animal feed.

The question if it is possible to alter the size and weight of a crickets' internal organs and intestines by using different feed could sadly not be investigated due to high mortality among the crickets' in the study. The reason for the high mortality rate is unknown. One possible cause could be virus infection. Other causes might include high stress levels or difficulty to regulate body temperature due to lack of god hiding places since plastic pots were used instead of the more commonly used paper cardboard egg holders. Little is known about the significance of good hiding places. Since mortality was high in both feeding groups (chicken feed and silage) the diet is not considered to be the cause. Worth mentioning though is that the Cambodian field crickets *(Teleogyrus testaceus)* fed weeds and cassava tops, which can be assumed to have a higher crude fibre content than chicken feed, did weigh more in comparison to their body size than the chicken feed group (Table 11). One possible explanation could be that the intestines of the crickets fed cassava and weeds were enlarged due to the roughness or difficult digestibility of the feed compared to chicken feed. This phenomenon has, as mentioned in the literature review, been observed in birds.

Using CT-scanning to evaluate the size and volume of a cricket's gastrointestinal tract is a technique in need of more research and refinement to be reliable. Due to a cricket's exoskeleton and the summation it creates it is necessary to use contrast to clearly see the gastrointestinal tract. To get a clear image of the entire gastrointestinal tract all parts must contain contrast which might be difficult to achieve. In live animals the movement of the intestines constantly move the contrast ahead and injecting contrast into dead crickets will be difficult without damaging the delicate structures. If the purpose of using the technique is to compare the volume and size of several crickets' intestines the entire intestine has to contain contrast to create comparable images of the different individuals. Considering the amount of time and work it took to prepare the scanning and to process the images this method might prove to be rather cost-benefit-inefficient. Examine crickets trough micro-dissection is likely to give more information to a lower cost.

In conclusion, this study support Miech *et al.* (2016) view that especially Cassava tops but also *Cleomone rutidosperma* have great potential to be used as feed for Cambodian field crickets. It is possible to use CT-scanning to produce an image of part of the gastrointestinal tract using contrast but more research is needed to develop the method if it is to be used to estimate the volume of the entire gastrointestinal tract on several crickets as it was a delicate and time-consuming task. The question if it is possible to alter the size and volume of a cricket's gastrointestinal tract by feed still remains unsolved.

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